


Evolutionary history of the birds of the Angolan highlands – the missing piece to understand the biogeography of the Afromontane forests

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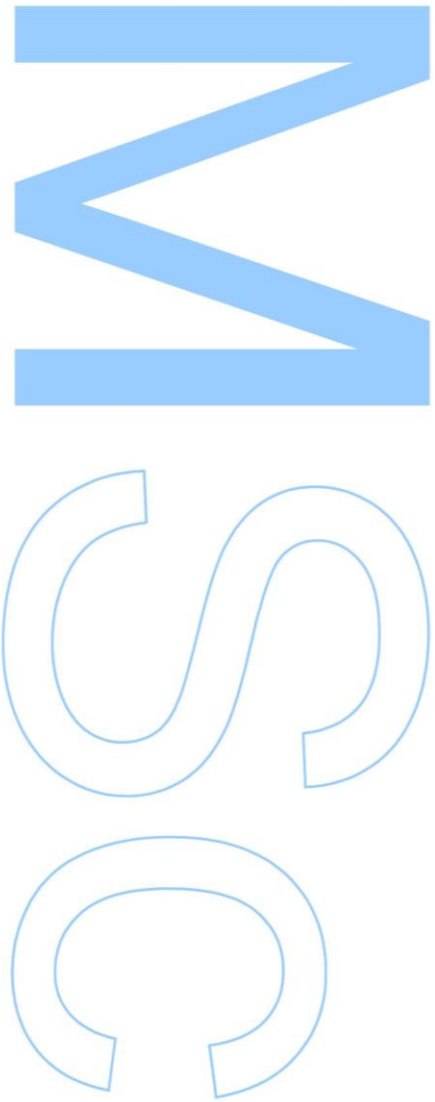

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Abstract

The similarities and differences in the avifauna of the montane forests of Africa have fascinated the ornithologist community throughout the last decades, but the study of the processes behind these distribution patterns has been conditioned by the lack of genetic data and robust analytical techniques; even the development of clear biogeographic hypotheses has been hindered by the lack of phylogenetic information for the avifauna of several important centres. Adding to this, these highland forests are at the imminent risk of disappearing, due to their restricted areas nested within regions with large human population densities and their predicted susceptibility to climate change, which make them both one of the most diverse and threatened habitats in Africa.

Recent research based on molecular methods has allowed the discovery of new species among the relatively well-studied East African montane forest bird communities, such as in the Eastern Arc, but important areas have yet to be studied. This is particularly true of the Angolan highlands, the focus of this project - a very important centre of bird endemism of particular interest because they are the most isolated “islands” of the Afromontane “archipelago”.

A phylogenetic, phylogeographic and niche modelling approach was used to contextualize the Angolan highland forests within the biogeographic history of African montane areas. This allowed, for the first time, to develop molecular-based models of the historical links between the major highland biodiversity centres in Africa. This study focused on five core species occurring in the Angolan highlands and in several highland centres throughout sub-Saharan Africa. All approaches showed that Angola played a fundamental role both as an ancient, very stable refuge from where isolated species could recolonize other centres during favourable climatic periods, and as a stepping-stone connecting the East Africa and the Cameroon Centres.

Keywords

Refugia, Divergence Times, Past Niche Reconstitution, Afromontane Regions

Resumo

As semelhanças e diferenças na avifauna das florestas de montanha de África têm fascinado a comunidade de ornitólogos ao longo das últimas décadas, mas o estudo dos processos por trás desses padrões de distribuição tem sido condicionado pela falta de dados genéticos e técnicas de análise robustas; até mesmo o desenvolvimento de hipóteses biogeográficas tem sido dificultado pela falta de informação filogenética para a avifauna de vários centros importantes. Para além disto, estas florestas correm o risco eminente de desaparecer devido a estarem incluídas em regiões com grandes densidades populacionais humanas e à sua prevista suscetibilidade a alterações climáticas, o que as torna num dos habitats simultaneamente mais diversificados e ameaçados em África.

Investigações recentes baseadas em métodos moleculares têm permitido a descoberta de novas espécies entre as comunidades de aves das florestas de montanha relativamente bem estudadas do Leste Africano, mas áreas importantes ainda necessitam de ser estudadas. Isto é particularmente verdadeiro para as terras altas de Angola, o foco deste projeto – um centro de endemismo de aves muito importante e de particular interesse por serem as "ilhas" mais isoladas do "arquipélago" Afromontano.

Uma abordagem filogenética, filogeográfica e de modelação do nicho foi usada para contextualizar as florestas de montanha de Angola dentro da história biogeográfica das montanhas Africanas. Isto permitiu, pela primeira vez, desenvolver modelos, de base molecular, dos elos históricos entre os principais centros de biodiversidade montanhosos em África. Este estudo focou-se em cinco espécies que ocorrem nas terras altas de Angola e em vários centros montanhosos ao longo da África Subsariana. Todas as abordagens mostraram que Angola desempenhou um papel fundamental quer como um antigo e estável refúgio de onde espécies isoladas poderiam recolonizar outros centros durante períodos climáticos favoráveis, quer como um local de passagem conetando o Leste de África e os Centros dos Camarões.

Palavras-chave

Refúgio, Tempos de Divergência, Reconstrução do Nicho Passado, Regiões Afromontanas

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Introduction

1. The Afromontane Archipelago

Mountains are usually viewed as impoverished environments in terms of biota due to their typically artic climate and rugged landscapes (Martin and Wiebe 2004). Nevertheless, it has recently been realized they play an important role in the generation of biodiversity. They contain half of the world's biodiversity hotspots even though they only cover 16.5-27% of the land area (Kohler and Maselli 2009). The high quantity of endemic and threatened species present in these environments makes them high priority areas for conservation (Stattersfield et al. 1998). Because of their climatic stability and, therefore, ability to maintain stable moist forest habitat, the high altitude areas of some tropical mountains have functioned as important refugia for many species throughout the different periods of climate fluctuation (Fjeldså et al. 2012; Weber et al. 2014).

In Africa, most of the continent is composed of a flat plateau of ancient rocks, which had very few areas of relief until the mid-Tertiary (Grimshaw 2001). The mountains and mountain ranges that can be seen today resulted from the intense volcanic and tectonic activity that initiated in the Miocene (Griffiths 1993, Lovett 1993). Moist forests associated with these mountains share more floristic components among themselves, even when separated by thousands of kilometers, than with the adjacent lowlands. As such, they have been classified as comprising an independent floristic region (White 1971). Because of the high fragmentation of this habitat due to their association with disjunct mountains, this floristic region was coined as the 'Afromontane archipelago' (White 1971, 1978; Grimshaw 2001). In this view, Afromontane forests are seen as occurring on isolated 'sky islands', lying in an 'ocean' composed of the inhospitable habitat of the African lowlands (Grimshaw 2001). There are seven traditionally

recognized areas of montane endemism in Africa (Fig. 1): the Cameroon Highlands, Angolan Highlands, Eastern Arc, Albertine Rift, Kenyan Highlands, Ethiopian Highlands and the Malawi (Tanganyika) Rift and southern Africa (Moreau 1966, Dowsett 1986). Recent analyses were unable to classify the Afromontane forests as an independent region taking into account other statistically defined biogeographical regions (Linder et al. 2012). Although they present clearly differentiated flora and fauna from the surrounding lowlands, the failure of objective analytical methods to retrieve the Afromontane Region can be explained due to their small size, which dilutes the contribution of their unique endemic communities in the surrounding lowland biota given the pixel-based (e.g. 1 degree square) data matrices used (Linder et al. 2012).

When it comes to birds, the high diversity of Afromontane centres together with their high specificity have always fascinated ornithologists, even though data on the genetic structure of Afromontane birds remains poor because samples are often unavailable for many areas. This prevents making broad scale studies looking at the Afromontane forests as a whole (Kahindo et al. 2007).

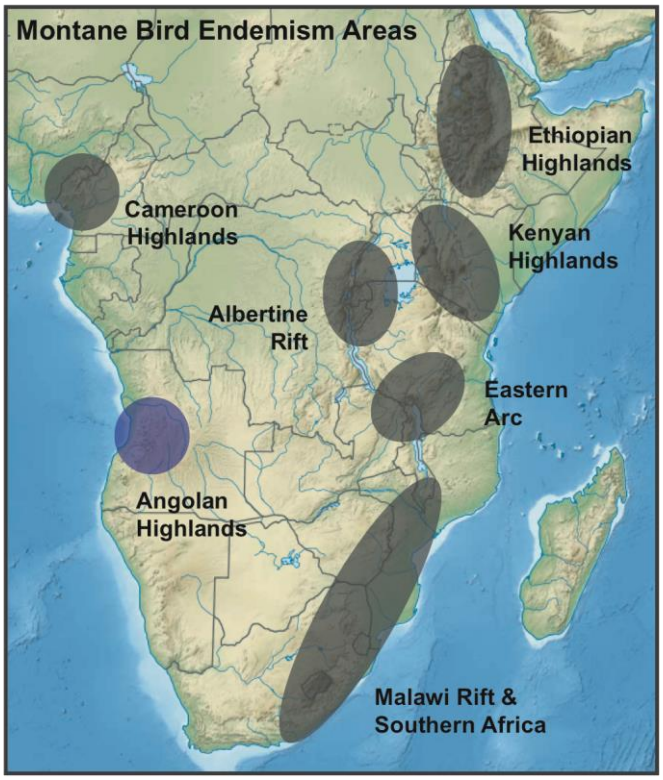

Figure 1 Africa's seven traditionally recognized areas of montane bird endemism: Cameroon Highlands; Angolan Highlands; Eastern Arc; Albertine Rift; Kenyan Highlands; Ethiopian Highlands; Malawi Rift and Southern Africa.

2. Climatic Cycles and Forest Dynamics

During the Quaternary, changes in temperature with very different amplitudes occurred throughout the world (Denton et al. 2010). These climatic shifts had a profound impact on the climates of Africa. It is generally believed that during the dry and cold periods of the glaciations in the Northern Hemisphere, the Southern Hemisphere's moist tropical forests retracted to higher elevations, expanding subsequently. This pattern has been described for Africa (Hamilton and Taylor 1991; Shi et al. 1998) but the situation may have been more complex and some authors have inferred the opposite pattern (Moreau 1966; Michel et al. 1993; Shi et al. 2000; Stuut et al. 2002). In this scenario, the decrease in temperature during the glacial periods led to an increase of the effectiveness of the rainfall due to the reduction in evaporation. Hence, it was during glacial periods that the forest belt expanded and communication was established between the montane habitats from the west and east of Africa, which are nowadays found in isolated and small areas. In summary, there is still no consensus about the tropical African climate history, but the most recent studies point to a scenario of humid conditions with increased rainfall during the glacials, related with the closeness to the equator (Shi et al. 2000; Stuut et al. 2002).

During the peak of the dry conditions followed by the extension of deserts and savannah, moist forests were restricted to humid micro-climate of the mountain areas, which provided a stable moist habitat throughout the interglacials. It has therefore been hypothesised that Afromontane forests acted as refuges from where the lowlands were recolonized during forest expansion phases (Mayr and O'Hara 1986; Dimitrov et al. 2012). In spite of this, these tropical forests contain not only ancient species but also clusters of recently diverged lineages, and they acted therefore also as centers of speciation due to their long-term stability (Fjeldså and Lovett 1997; Fjeldså et al. 2005). This has for example been demonstrated from the divergence in mtDNA in greenbuls from east Africa (Roy 1997).

As mentioned before, Afromontane areas are known to have a very different flora and fauna from those of the surrounding lowlands. If we look at the Afromontane fauna, the stability of the montane forests throughout the Pliocene was enough to allow different species to persist despite the stark transition from forest to savannah during the drier periods. This isolation in the high peaks allowed species, such as the *Kinyongia* chameleons (Tolley et al. 2011), to speciate into different neooendemic species in different Afromontane islands through the preservation of ancient lineages, right after major episodes of lineage diversification permitted by the forest re-expansion and connectivity during climate optima (Zachos et al. 2001, Couvreur et al. 2008).

The vegetational/climatic cycles of the Pleistocene, with the formation and degradation of habitat corridors between the forest cores, is also believed to have affected patterns of speciation in more mobile organisms, like the passerine birds. It remains uncertain whether these Pleistocene cycles caused increased speciation or the opposite. There is support for the refuge hypothesis that isolation (fragmentation) of montane forests has facilitated speciation in birds (Mayr & O'Hara 1986); however, it is known that many speciation events happened well before the Pleistocene, making the Pleistocene refugia hypothesis not appropriate as a general model for explaining patterns of Afromontane bird diversification; rather, both dispersal and vicariance might also have played important roles in shaping montane bird communities (Fjeldså & Bowie 2008).

3. The Human Threat to Dispersal

As Grimshaw (2001, p. 951) puts it: 'Where does the Afromontane archipelago actually begin?' The distinction between montane and lowland vegetation is based on the ideas of Greenway (1973) and White (1983) that use a phytochorological delimitation, but this question is a difficult one to answer because of the destruction of the transitional vegetation on the lower slopes of the mountains due to the need of the local human communities for wood and arable land (Grimshaw 2001). These areas, together with the highland forests, are disappearing at a worrying rate and the overwhelming majority still remain outside of protected areas networks, which make them in urgent need of study before they may disappear forever. Burgess et al. (2007) and Fjeldså et al. (2008) showed that there is a positive correlation between species richness and endemism and human population density. From a conservation point of view, this could lead conservationists to focus not only on wilderness areas with few people, but also on developing strategies for sustainable development in the densely populated areas.

The problem with the Afromontane bird community is the extreme dispersal limitation amongst different species. In the absence of corridors connecting the disjunct Afromontane areas, maintenance of avian diversity is hardly possible, which has led to local extinctions (Moore et al. 2008). If unregulated and unsustainable human exploration and expansion continues to destroy the essential landscape between the isolated Afromontane areas where the dispersal corridors were formed in the past, forest-restricted species might not be able to disperse and diversify in the future.

4. The Angolan Highlands

The most isolated Afromontane centre of endemism is precisely in Angola, being separated by more than 2000 km from other Afromontane islands such as the ones in the Eastern Arc (one of the best studied Afromontane hotspots). This high isolation gave rise to distinct and unique bird communities (White 1981; Dowsett 1986; Grimshaw 2001) but an important component of the forest restricted bird community found in Angola is shared with other distant Afromontane centres. The Afromontane forests in Angola constitute the second most important habitat of the country's only Endemic Bird Area (Collar and Stuart 1988). They occur in isolated patches in the high peaks of the provinces of Benguela, Cuanza Sul, Huambo and Huíla. Until recently, it was believed that all these forests covered less than 200 ha of area, making them the most threatened habitat in Angola (Huntley & Matos 1994; Stattersfield et al. 1998). The recent discovery of a large Afromontane forest (c. 590 ha) on the Namba Mountains was therefore very significant (Mills et al. 2013). This mostly continuous patch is much less susceptible to edge effects and is less affected to human impacts due to the ruggedness of the terrain; it shows an almost pristine environment, bearing 20 bird taxa of conservation significance (Mills et al. 2013).

Mount Moco (Huambo), Angola's highest mountain, encompasses the best-studied montane forests in the country (Mills et al. 2011). They host several endemic and endangered birds, such as the flagship Swierstra's francolin (*Pternistis swierstrai*), which makes this a critical area from a conservational perspective (Huntley 1974, Mills et al 2011). Nevertheless, only 85 ha of forest, fragmented in very small patches, remain due to the unsustainable use of forest resources and human-caused fires (Huntley & Matos 1994, Mills et al 2011).

According to Dowsett (1986), when looking specifically at the forest bird list there is a strong correlation between the species occurring in Angola and the ones in east Africa, regardless of the massive distance between these two areas when comparing to the proximity of Angola to Cameroon. This link between Angola and East Africa was likely established by a corridor via Zambia, where today some small montane forest patches still occur (Dowsett et al. 2008).

5. Modelling the species distribution history

Today, molecular tools, the development of large digital distributional databases and the modelling of past habitat conditions gave rise to a new era of Biology, making it possible to attempt to unravel the underlying process leading to present-day observed patterns of spatial distribution easier and faster than ever before (Fjeldså 1994; Fjeldså

& Bowie 2008). The identification of the past potential distributions of bird species can provide a valuable spatial/temporal framework to understand and corroborate the results from phylogeographic approaches. The distribution and diversity of haplotypes across space can indicate historical refugia, patterns of differentiation and similarity and identify events of isolation. However, extinction of key haplotypes, incomplete sampling and range shifts can mask our perception about past distributions. Fossil distributional data could provide a proxy to solve some of these biases but this is usually difficult with small, fragile animals, such as passerine birds.

The use of ecological niche models (ENMs) in conjunction with paleoclimatic reconstructions can provide valuable data to help infer past distributions (Waltari et al. 2007). These kinds of models quantify the spatial suitability using presence data and the most important environmental parameters that shape each species ecological niche. They assume that the current distribution is mainly determined by the environmental parameters and interspecific factors (e.g. competition) and that dispersal limitation is negligible. Although the robustness of the model is easier to test under present environmental conditions, it is possible to use past environmental parameters based on paleoclimatic reconstructions to reconstruct past ecological niches. Although this assumes niche conservatism with negligible niche shift, this approach has proved valuable in bird species where the most important environmental parameters are conditioned by geographic variables such as altitude.

6. Objectives

The major aim of this work was to provide a clear picture of the relationships between the Angolan centre of montane bird endemism and other African mountain forest cores by analysing and combining, for the first time, empirical information from gene evolution and circumstantial information from past habitat evolution. Ultimately, by integrating our and previous results of the different species that were exposed to the same evolutionary forces, we were able to perform a comparative analysis of the phylogeographic patterns. From a conservation perspective, this provides robust past and present data to identify environmental settings more likely to help maintain the evolutionary process into the future (Mace & Purvis 2008).

The first objective was to perform a comparative phylogeography of several Afromontane species present in Angola.

In order to better understand the evolutionary history of these species, the second objective was to describe the niche occupied by them in the present days (climate/vegetation cover) and to reconstruct the climate/vegetation of the past in order

to understand where fragmentation occurred, and what were the more stable, long-lasting, habitat corridors between the now isolated forest cores.

Methods

1. Area of study and Sampling

In this study, five African passerine model species (Fig. 2). were selected because they are known to occur in the Angolan highlands and are widely distributed throughout other montane areas of sub-Saharan Africa, serving as good model species to understand the pathways between Angola and other Afromontane hotspots.

Samples were obtained mainly from captured birds using mist nets in suitable habitats in the different Afromontane systems. Blood samples were obtained from the wing vein by venipuncture and mainly stored in ethanol. The blood samples were stored in our research center for later DNA extraction. The samples used in this study resulted from several years of sampling effort (2003 - 2014) by Martim Melo and collaborators. Additionally, several blood and tissue samples obtained by other researchers were extracted by collaborators in the University of California, Berkeley (see Attachment 1).

2. Comparative Phylogeography

The genetic information was retrieved either in the laboratory, using GenBank or provided by our collaborators, and then analysed using classic population genetic and phylogenetic tools.

2.1 DNA sequencing

Total genomic DNA was extracted from blood samples previously preserved in ethanol at -20ºC using a Genomed JetQuick (250 samples) kit, following the protocol for DNA extraction from normal blood samples. PCR was used to amplify two mitochondrial genes: NADH dehydrogenase 2 (ND2: 1041 bp) and ATPase 6 (ATP6: 684 bp).

Primers and PCR conditions for these markers are detailed in Attachment 2. The products from the PCR were checked by electrophoresis in agarose gel and then purified using EXOSAP (Exonuclease I and Shrimp Alkaline Phosphatase). PCR products were sequenced in both directions by Macrogen Inc, Amsterdam, Netherlands, using the EZ-Seq service.

All sequences were edited in the software Geneious R8.1.6 (Biomatters Ltd., http://www.macrogen.com/). The forward and reverse sequences from each individual obtained in the previous step were assembled, trimmed and inspected by eye to correct double peaks. In the rare cases of the double peaks being dubious, each base was coded as missing data (assigned as "N"). The consensus sequence obtained from both forward and reverse sequences for each individual was extracted and all consensus sequences plus the sequences obtained from the other sources were aligned using the MAFFT Alignment plug-in (Katoh 2013). All alignments were again inspected by eye to check for insertions, deletions and gaps, and concatenation of both ATP6 and ND2 alignments was performed. The missing data in the final concatenated alignment was filled with "Ns" in order for all sequences to have the same length.

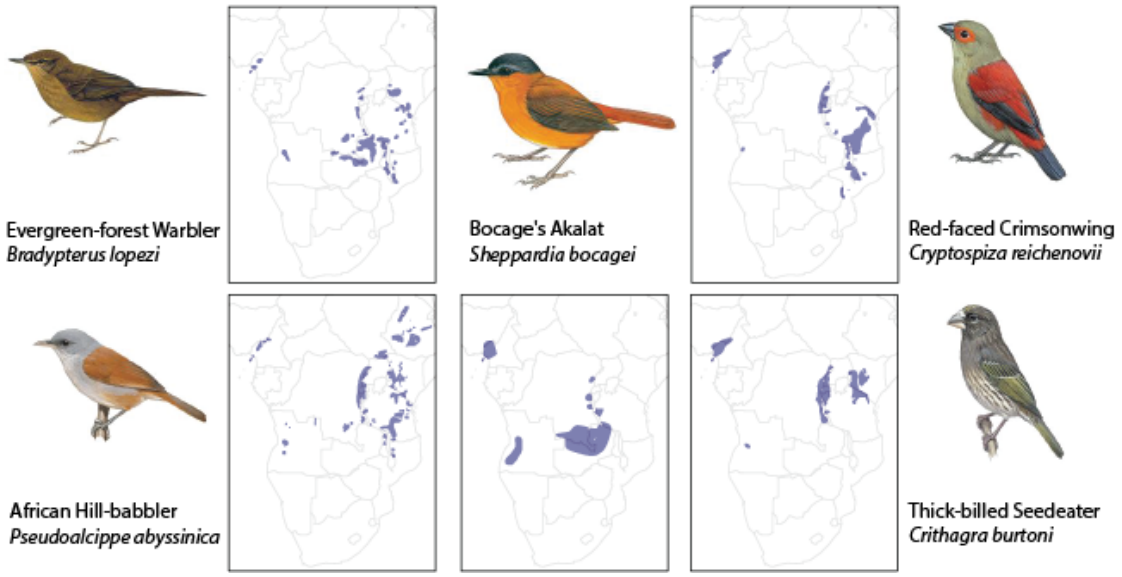

Figure 2 The five selected species and their current distribution.

2.2 Divergence times calculation

The software BEAST v1.8.2 (Bayesian Evolutionary Analysis Sampling Trees) (Drummond et al. 2012) was used to estimate the divergence times of the different clades. The best partition for the two-genes dataset was determined using PartitionFinder v1.1.1 (Lanfear et al. 2012). A single model - GTR (Tavaré 1986) with Gamma + Invariant Sites - was applied to the entire dataset. The Yule process (Yule

1925; Gernhard 2008) was used as the tree model. Divergence times were estimated both under a strict clock and a lognormal relaxed clock. The clock rates applied for each loci were those estimated by Lerner et al. (2011) for the Hawaiian Honeycreeper radiation using a normal prior distribution (mean of 0.026 s/s/l/my for ATP6 and a mean of 0.029 s/s/l/my for ND2, both with a stdev of 0.003 and a starting value of 0.02). Two independent runs for each model clock were carried out – each of 25 millions of generations, sampled every 2500 generation (10000 samples per run); 25% of the samples were discarded as burn-in. The effective sample size (ESS), after burn-in, for each parameter of interest in the analysis was evaluated using Tracer v1.6.0 (Rambaut et al. 2014). The samples from the independent runs were combined with LogCombiner v1.8.2 (Drummond et al. 2012). TreeAnnotator v1.8.2 (Drummond et al. 2012) was used to summarize the information in a sample of trees produced by BEAST and the resulting trees for each marker were visualized with FigTree v1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/), including the summary information produced by TreeAnnotator.

To corroborate the relationships inferred by BEAST, independent phylogenetic analyses were also performed with the alternative Bayesian approach implemented in MrBayes v3.2.5 (Huelsenbeck and Ronquist 2001; Ronquist and Huelselbeck 2003) and a maximum-likelihood search as implemented in RAxML v8.0.0 (Stamatakis 2014). The best data partition (defining all codon positions of each gene as a potential partition) and the best substitution model for each partition were estimated with PartitionFinder. The dataset was partitioned in two sets (first and second codon position; third codon position), both following a GTR+I+G substitution model. For MrBayes, two independent searches with four chains each were run for 10 million generations, sampled every 1000th generation. A 25% burn-in was applied, and confirmation that the searches had reached convergence was obtained from inspecting results in Tracer. For RAxML, 10 independent runs of a thorough maximum likelihood analysis were performed; support was calculated with 500 bootstrap pseudo-replicates.

3. Niche Modelling

To complement the results obtained from the phylogenetic study, species distribution modelling was performed. A set of bioclimatic variables (see Attachment 3) was used as independent variables to model current and past distribution of each species using a maximum entropy modelling approach (Elith et al. 2011). This model minimizes the relative entropy between two probability densities (one estimated from the presence data and the other from the covariates, i.e. the environmental parameters). This was

implemented using Maxent 3.3.3 (Phillips et al. 2004 & 2006, Elith et al. 2011), SDMTools and Climate R packages (VanDerWal et al. 2011a, 2011b) and custom R scripts (see supplementary materials) in R 2.2. (http://www.r-project.com/). We used bioclimatic variables (resolution 2.5 arc-minutes) for 64 time slices, ranging from the present time to the beginning of the last glacial period (130 kya). In more detail, these included the Last Glacial Maximum (LGM; 21 kya), the Last Interglacial (LIG; c. 135 kya) and 62 additional time slices representing 1000-year intervals from the present back to the last glacial maximum (22 kya), continuing with 2000-year intervals until 80 kya, and then every 4000 years back to 120 kya.

Present bioclimatic variables were available at the WorldClim database server (http://www.worldclim.org/) from interpolations of observed data from 1950-2000. For past conditions, bioclimatic variables were derived from downscaled climate data from simulations with Global Climate Models (GCMs). In more detail, for each time slice, past climates were based on a snapshot simulations at up to 1 kyr intervals covering the last 120,000 years using the Hadley Centre Climate model (HadCM3) (Singarayer & Valdes 2010). Monthly temperature and precipitation anomalies were downscaled using a bilinear spline to 0.2 degrees and then bicubic spline to 0.0466667 degrees globally, and the anomalies were then applied to a current monthly climates given an '125 m lower sea levels' provided by Robert Hijmans (unpublished data; methods as per Hijmans et al. 2005). Past sea levels were estimated as the consensus of three sources: Lea et al. (2002), Robert A Rohde (unpublished data, Global Warming Art project available at http://en.wikipedia.org/wiki/File:Post-Glacial_Sea_Level.png and derived from Fleming et al. 1998; Fleming 2000; Milne et al. 2005) & http://www.ncdc.noaa.gov/paleo/ctl/clisci100k.html#sea. Mean annual temperature, temperature seasonality, mean temperature of the warmest and coldest quarters, mean annual precipitation, precipitation seasonality and precipitation of the wettest and driest quarters were recreated for each time into the past. All climate surfaces were clipped to 'dry land' based on sea levels. All downscaling and recreation of climate surfaces were done using the climates package (VanDerWal et al. 2011a) and R 2.9.0 (http://www.r-project.com/). All this data was clipped to a region of Africa that included all the potential Afromontane regions and the study species distribution.

The models used present day records of the presence of each species in the same spatial resolution as the bioclimatic variables. These were compiled from observation and ringing records, museum and genetic vouchers from several sources, including eBird (http://ebird.org), GBIF (http://www.gbif.org/), Observado (http://observado.org/) and Lubango Museum. The data was curated to remove duplicates and dubious

records (e.g. records with higher geographic uncertainty that the cell size) and filtered so that only one observation per cell was used, to avoid spatial biases.

Using these variables, each species potential niche was quantified for each time slice as a continuous probability values (ranging between 0 and 1), an indicator of relative suitability for each cell. Each model was run using a convergence threshold (nn), a maximum number of iterations and using a percentage of records for model training.

The overall species suitability for each cell was summarized in order to infer areas where climate variability had the least impact during the studied time period (i.e. stable areas) and also to infer the areas that showed less resistance to dispersal between the current isolated Afromontane regions. This was performed using two approaches (static and dynamic refugia). For the static models a consensus stability raster assuming no dispersal was obtained by summing the formula of suitability through time for each individual cell of the raster. For the dynamic approach, dispersal was incorporated into the model to allow a species to disperse to other cells with suitable habitat before going locally extinct. This was run for dispersal distances at 10 m increment intervals, from 10 to 70 m per year.

Results

1. Phylogenetic analysis

The current potential distribution of the five species in this study can be divided in three distinct and isolated Afromontane areas – the Angolan highlands, the Cameroon centre (including Bioko Island and Nigerian mountains, part of the Cameroon Line of Volcanoes) and the Eastern Archipelago (comprised by several islands present in the Democratic Republic of the Congo, Kenya, Malawi, Mozambique, Tanzania, Uganda and Zambia).

In general, the runs performed in MrBayes and RAxML inferred the same phylogenetic history as the long run performed in BEAST, with each software providing high support values (≥95% for Bayesian inference – BEAST/MrBayes; ≥85% for maximum likelihood bootstraps - RAxML) for the major branches with few exceptions (Figs. 3a-c). Different species presented distinct phylogenetic histories, discarding the hypothesis that all of them were affected in a similar way by the forest changes driven by the climatic cycles. Three different scenarios were recovered. The first can be observed in the phylogenies of *Bradypterus lopezi* and *Cryptospiza reichenovii* (Fig. 3a), where Angola samples group with the Cameroon centre. The second occurs only in the *Crithagra burtoni* tree (Fig. 3b), where Angola occurs amongst the Eastern Archipelago haplotypes. The third appears on the *Pseudoalcippe abyssinica* and *Sheppardia bocagei* trees (Fig. 3c), where the Angola haplotypes are basal and isolated from all the other samples.

Divergence time estimates were not affected by the model clock used (strict or relaxed lognormal). Divergence times obtained for the full dataset (all species, the two genes) were also very similar to estimates obtained from independent runs per species and per gene.

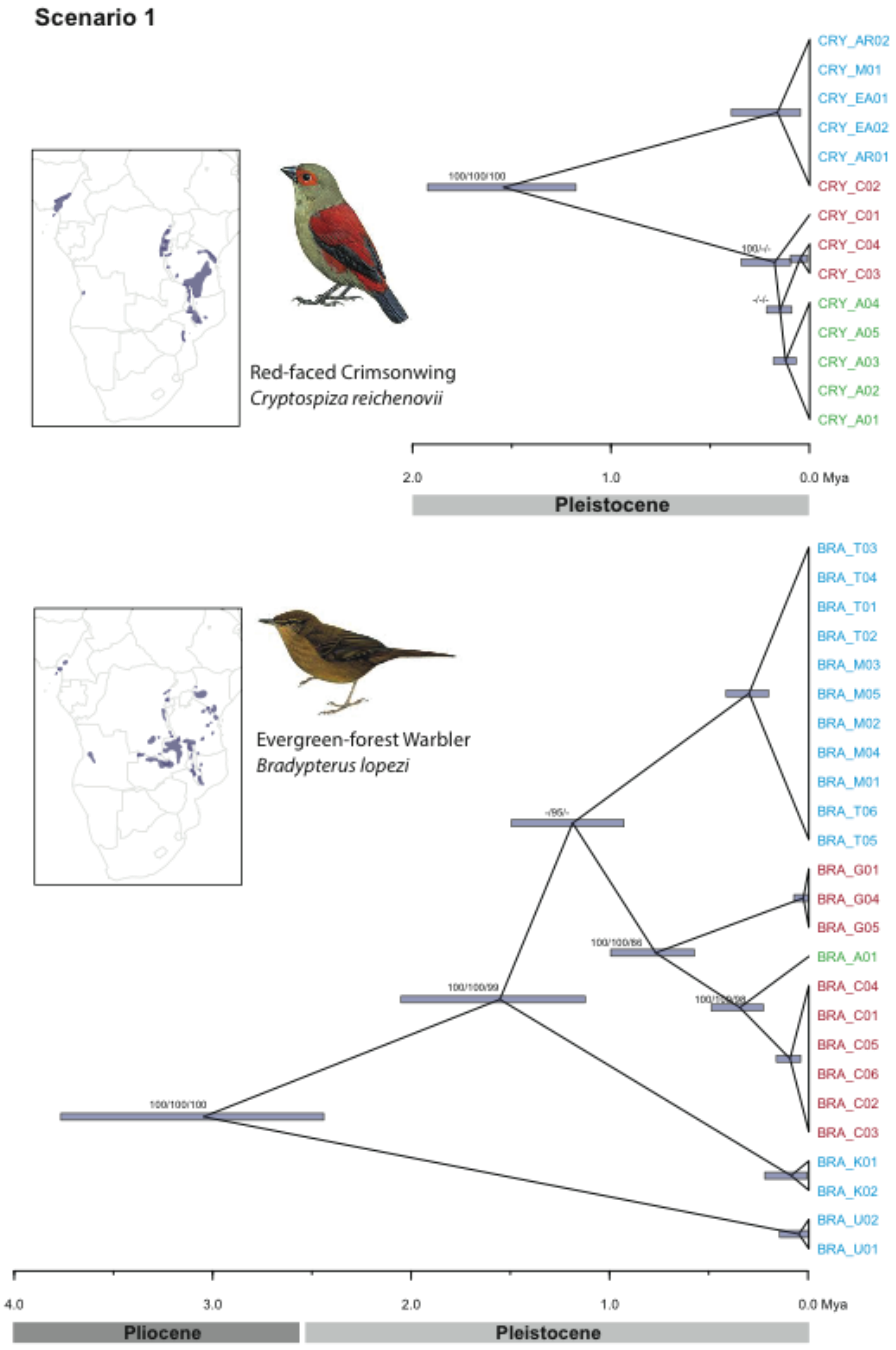

Figure 3a Phylogenies for *Bradypterus lopezi* and *Cryptospiza reichenovii*, based on the concatenation of ATP6 and ND2 markers, analysed with Bayesian inference and Maximum Likelihood. Bayesian posterior probabilities values ≥95% and ML bootstrap values ≥85% are presented at the nodes by the following order - BEAST/MrBayes/RAxML. Confidence intervals for the divergence times are marked with blue bars. In blue the haplotypes from the east, in red the haplotypes from the Cameroon centre and in green the haplotypes from Angola.

In the case of *Bradypterus lopezi* (Fig. 3a), two early splits can be observed. First for the Uganda haplotypes (about 3.1 Mya) and then for the Kenya haplotypes (1.6 Mya), followed by the split between the other eastern haplotypes (Tanzania and Malawi) and the western ones (Cameroon, Equatorial Guinea and Angola) at about 0.8 Mya. In this

case, the only Angola haplotype that was available nests within the same clade as the Cameroon and Bioko Island ones, an evidence of a very recent split between Angola and Cameroon, at about 350 thousand years ago.

For *Cryptospiza reichenovii* (Fig. 3a), a split between the western and eastern haplotypes occurred at about 1.5 Mya. The eastern clade includes the haplotypes from individuals from Malawi, Tanzania, Albertine Rift but also and from a single individual from Cameroon,. The western clade incudes all the other individuals from Cameroon and the five from Angola. The Angola haplotypes diverge from two of the Cameroon haplotypes by only about 150 thousand years giving little phylogenetic signal and, therefore, little support to this split.

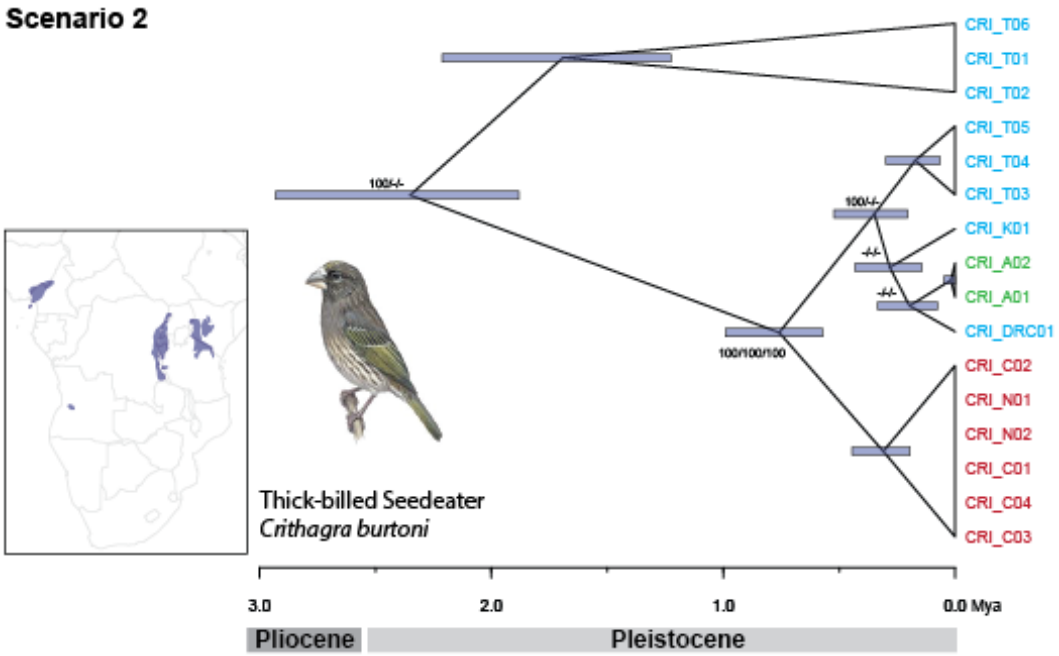

Figure 3b Phylogeny for *Crithagra burtoni*, based on the concatenation of ATP6 and ND2 markers, analysed with Bayesian inference and Maximum Likelihood. Bayesian posterior probabilities values ≥95% and ML bootstrap values ≥85% are presented at the nodes by the following order - BEAST/MrBayes/RAxML. Confidence intervals for the divergence times are marked with blue bars. In blue the haplotypes from the east, in red the haplotypes from the Cameroon centre and in green the haplotypes from Angola.

For *Crithagra burtoni* (Fig. 3b), the Tanzania haplotypes occur in two different clades: a basal one comprising exclusively three Tanzanian individuals (Ndundulu Mountains, Kisinga Lugalo, Udzungwa Scarp), and a more recent one comprising three Tanzanian individuals (Kisinga Lugalo, Nou Forest, Salanga Forest), two eastern haplotypes (Kenya and Democratic Republic of the Congo) and the two Angola haplotypes. The oldest clade split at about 2.4 Mya from the remaining ones, and the recent one split at about 770 thousands years ago from a clade grouping the northwest haplotypes of Cameroon and Nigeria.

The *Pseudoalcippe abyssinica* (Fig. 3c) samples from Angola are basal to all other samples from which they have diverged for about 3.5 Mya.. The separation between the eastern and the northwest haplotypes is not very clear in this case, with the Tanzania (Eastern Arc Mountains) and the Albertine Rift haplotypes diverging first (1.4 Mya and 1.0 Mya, respectively) but the Kenya ones occurring in the same clade of the northwest haplotypes (Cameroon and Bioko), presenting very low support values with MrBayes and RAxML for the split between these two areas.

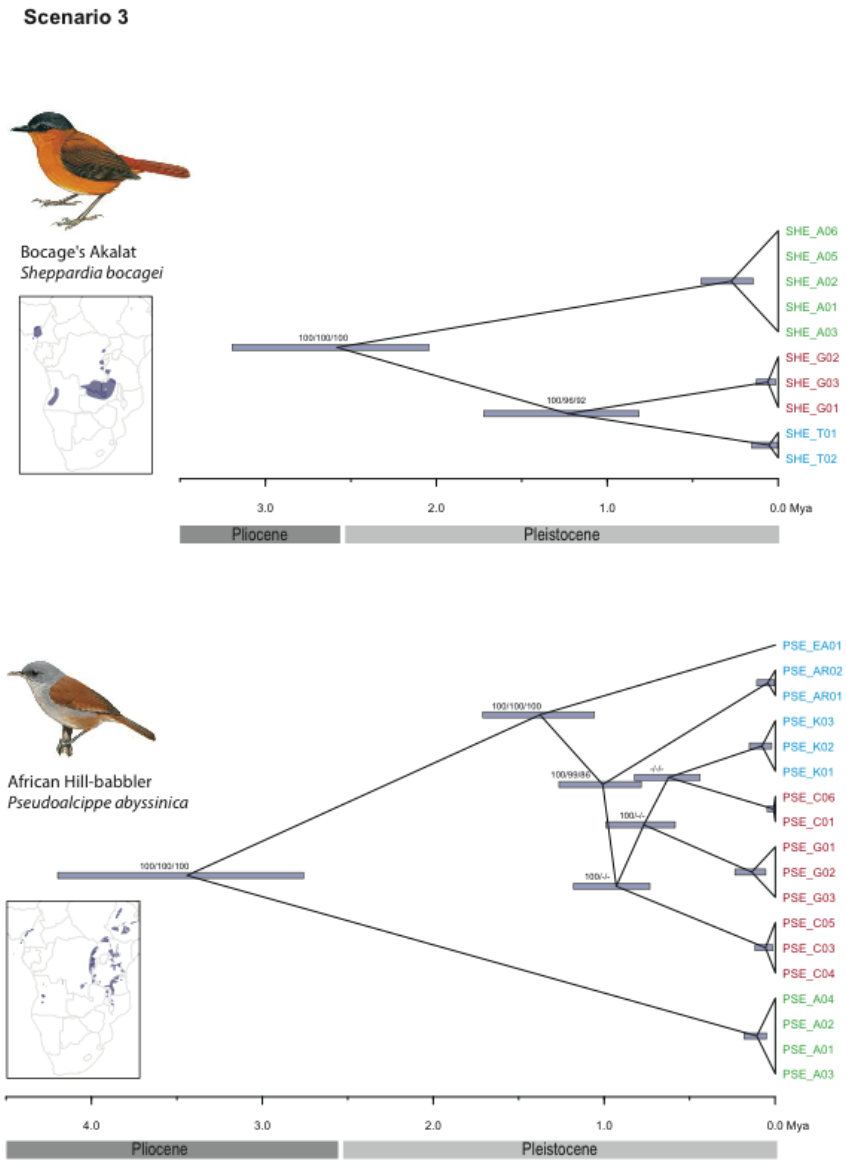

Figure 3c Phylogenies for *Sheppardia bocagei and Pseudoalcippe abyssinica*, based on the concatenation of ATP6 and ND2 markers, analysed with Bayesian inference and Maximum Likelihood. Bayesian posterior probabilities values ≥95% and ML bootstrap values ≥85% are presented at the nodes by the following order - BEAST/MrBayes/RAxML. Confidence intervals for the divergence times are marked with blue bars. In blue the haplotypes from the east, in red the haplotypes from the Cameroon centre and in green the haplotypes from Angola.

Sheppardia bocagei (Fig. 3c) is the only species for which there is a clear divergence between the three major centres (Angola, Cameroon, East Africa) with high support values from all the inference methods used and no episodes of recolonization detected. The Angola haplotypes were the first to split, at about 2.6 Mya, followed by the split between the Cameroon (Bioko Island samples) and the Eastern (Tanzania) centres, at about 1.3 Mya.

2. Past niche reconstruction

When projecting the SDMs to the Last Glacial Maximum (21 kya), the connection between the eastern islands becomes clearer due to an expansion in the distribution of the species niches (Fig. 4). It is also possible to observe strong effects across the central portion of the continent connecting Angola with the Eastern Archipelago, and reinforcing the hypothesis postulated from current species distributions of the importance of a corridor through Zambia during the glacials (especially in the case of *Sheppardia bocagei*). Corridors between the Cameroon center and both Angola and Eastern Africa were also formed during the most humid periods, but these do not seem to have been as large and stable/frequent.

Projecting the SDMs even further into the beginning of the last glacial period (c. 120 kya) a probability of occurrence scenario very similar to the present days can be observed as expected, showing very weak or even absent connections between these three major areas and still presenting the characteristics of an interglacial (Fig. 4).

When looking at the overall species suitability for each cell using the static refugia approach (Fig. 5) it is possible to infer that during the studied time period Angola and the Cameroon centre form the most stable centres in the case of *Sheppardia bocagei*. A high stability for these two centres can also be observed for *Bradypterus lopezi*, *Cryptospiza reichenovii* and *Pseudoalcippe abyssinica*, although in these cases the most stable areas occur in the Eastern Archipelago. In the case of *Crithagra burtoni*, it is possible to observe stable areas corresponding to the Cameroon centre and the Eastern Archipelago, but the stability of the Angolan centre is not very evident. It is also possible to observe suitable areas between Angola and the Eastern Archipelago in all five cases (especially in *Sheppardia bocagei* but not so evident in *Crithagra burtoni*), corresponding to the corridors formed during this glacial period. The occurrence of suitable areas between Angola and the Cameroon centre is not so evident and they are almost inexistent between the Cameroon centre and the Eastern Archipelago.

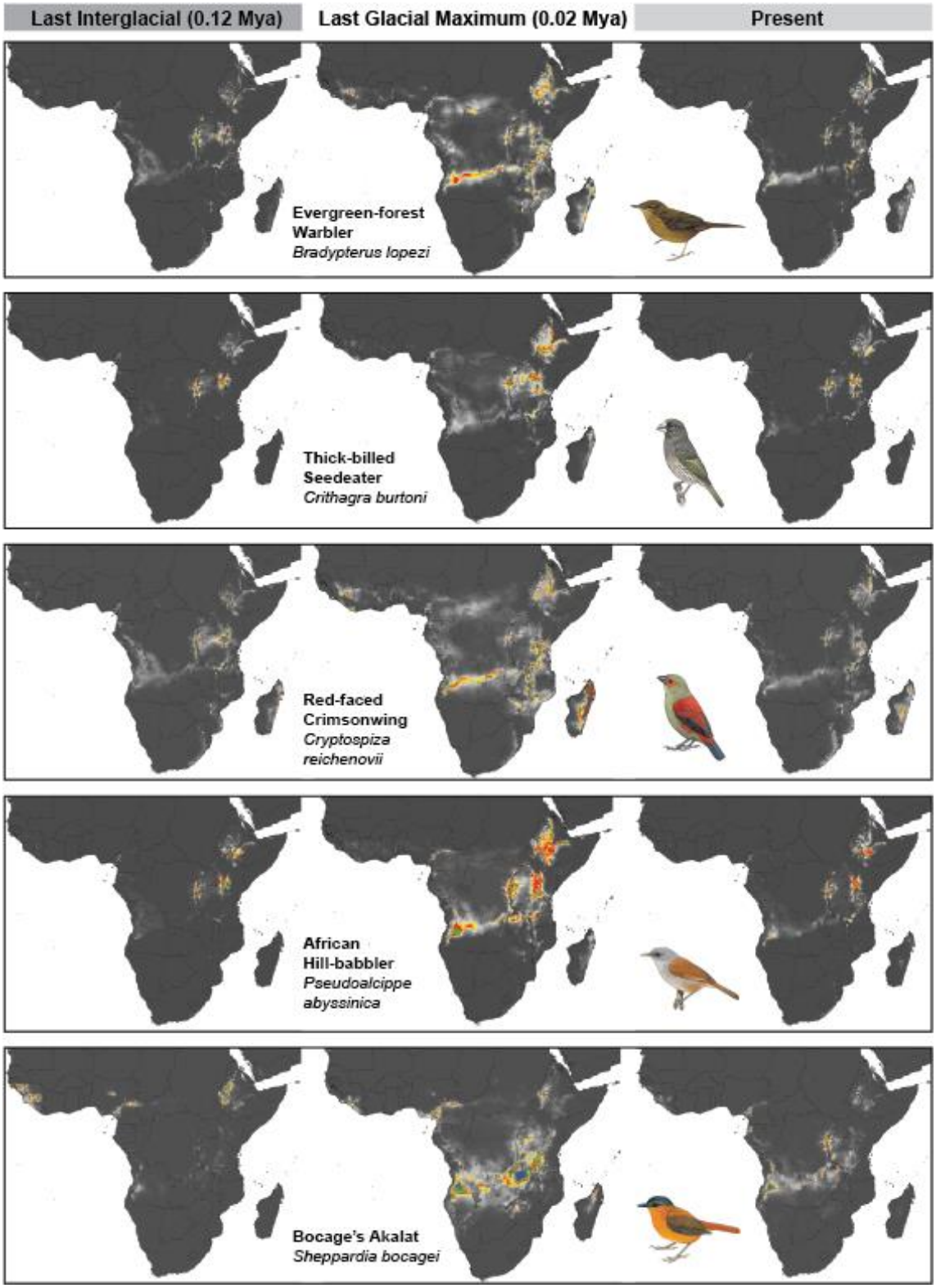

Figure 4 Projections for each species of the Species Distribution Modelling to the beginning of the last glacial period (120 thousand years ago), the Last Glacial Maximum (21 thousand years ago), compared with the projection for the present.

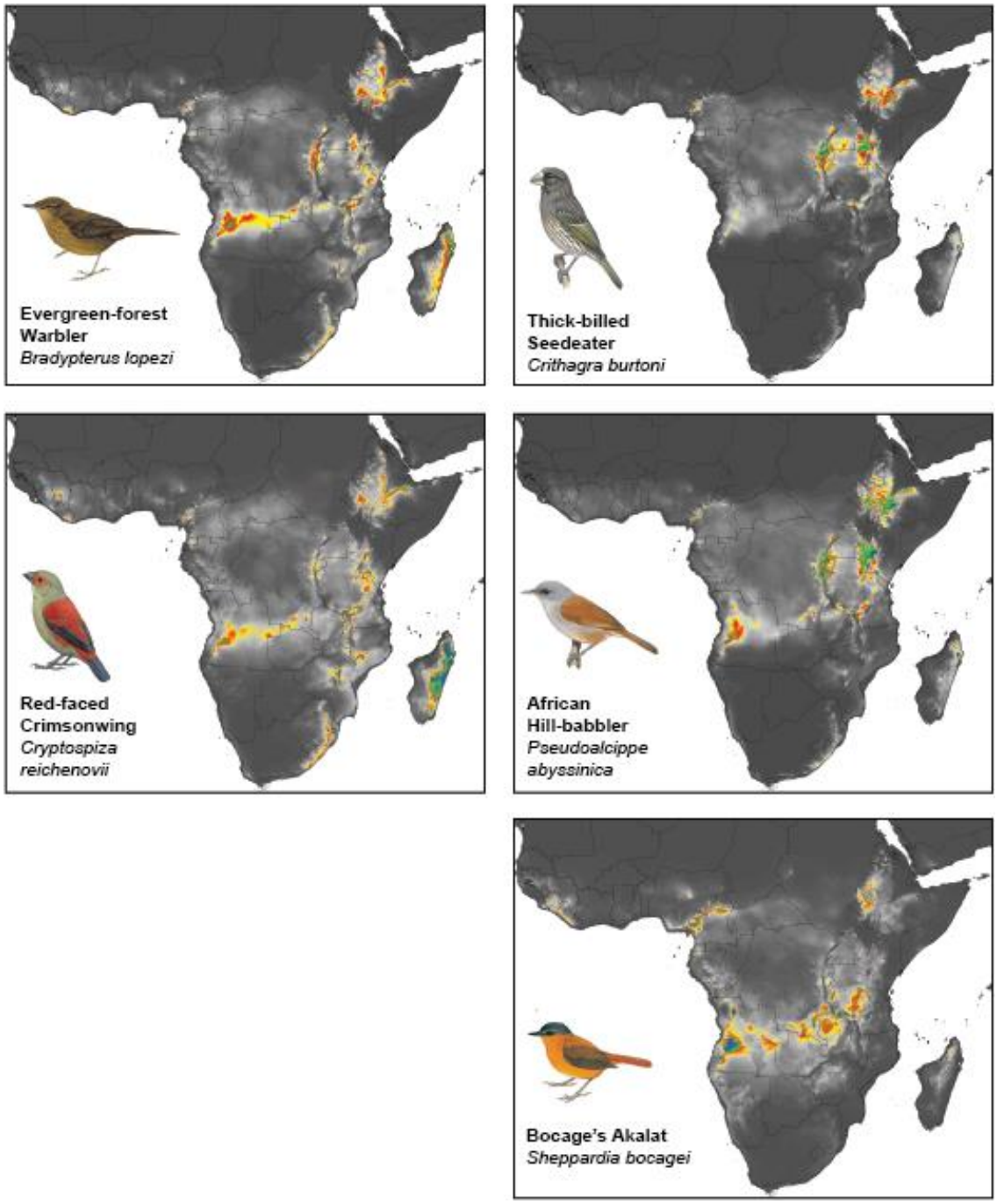

Figure 5 Overall species suitability for each cell using the static refugia approach.

When analysing the overall species suitability for each cell using the dynamic refugia approach (Fig. 6) the migratory routes already described through the corridors between Angola and the other two centres are more evident. However, there is no evidence of an increase of habitat suitability between the Cameroon centre and the Eastern Archipelago.

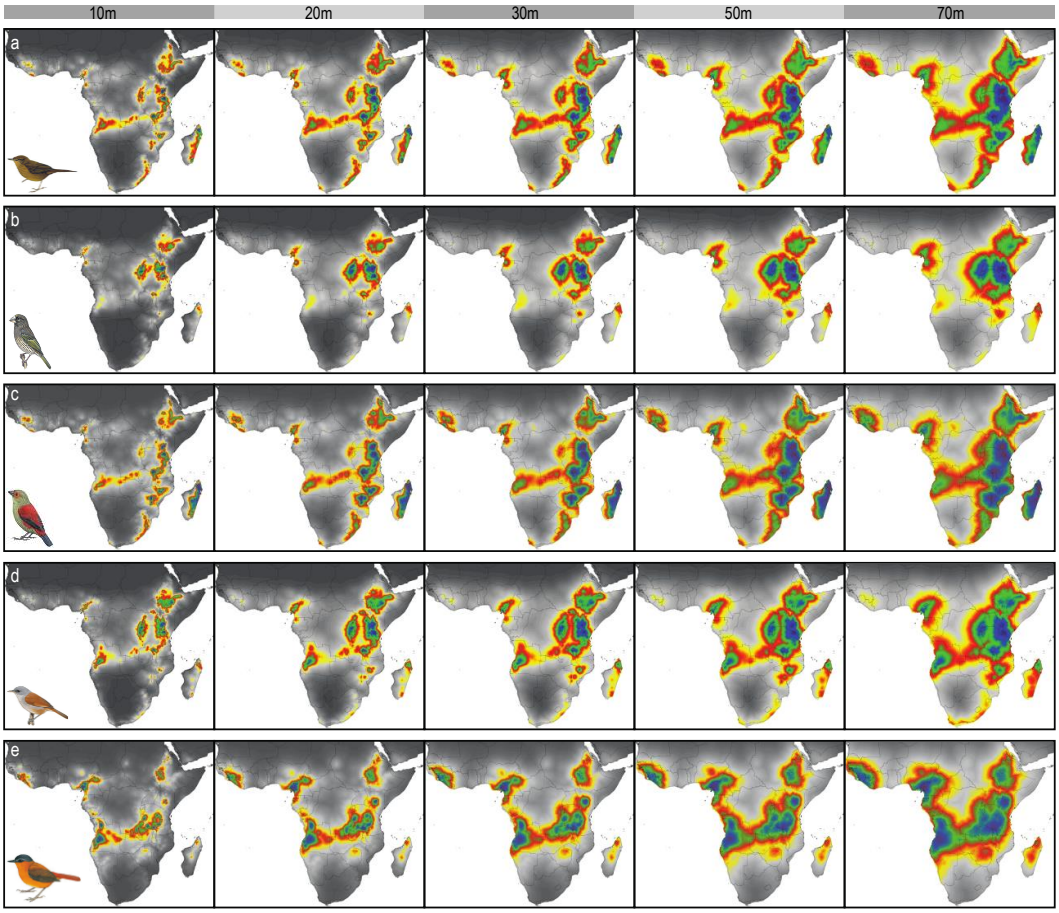

Figure 6 Overall species suitability for each cell using the dynamic refugia approach. (a) *Bradypterus lopezi*; (b) *Crithagra burtoni*; (c) *Cryptospiza reichenovii*; (d) *Pseudoalcippe abyssinica*; (e) *Sheppardia bocagei*.

Discussion

The moist forests from the highland centres of Africa constitute a fascinating example of ecological islands. This is particularly evident in the bird communities – although birds are known for their mobility, Afromontane centres thousands of kilometres apart share species that are not found in the habitats immediately adjoining the forests. How did the different Afromontane bird communities became isolated, i.e., in what order did links between different mountains become weaker before fading completely, is a question that fascinates biogeographers but which remains unanswered. One of the reasons is that there has been no studies across some pieces of the puzzle. The major missing piece so far has been the Afromontane centre of Angola – a small and very isolated centre, which nevertheless lies as a potential link between the Eastern and Western Afromontane centres. This study is the first to include Angola, and hence attempt to reconstruct the 'big picture' of the dynamics of disjunction and reconnection that have played a central role in the diversification and evolution of the Afromontane communities.

Our current knowledge on species distribution brings forward a close link between Angola and the Eastern African centres (Dowsett 1986), as many species or closely related species are shared between both. This link was also recovered with our modelling of the past 120 thousand years. For four out of five species, the most common and wide corridors were indeed those linking Angola to the East. Still, the evolutionary history inferred from genetic data for five species present in all major centres unveiled more complex, diverse relationships between the different centres. In fact, from the five model species only one clearly followed the pattern expected from a close link between Angola and East Africa. Our data recovered close links between Angola and the Cameroon centre, and perhaps more significantly, four species suggest

that Angola may be an old and stable Afromontane centre, from where other centres may have been recolonized.

Contradicting the first expectations of the existence of a common pattern of diversification amongst the five species studied due to their similar current distribution and genetic relatedness, the results show that each case has a different evolutionary history, although some commonalities are present. In spite of the SDM results in this study going back in time only to the beginning of the last glacial period (120 kya) and all of the splits inferred occurred before that period, one can safely assume that the conditions which allowed such splits in the remote past were similar as the ones included in the SDM results, as they were under the control of the same cyclic climate oscillations throughout the Pleistocene (Fig. 7). These periodic oscillations in the subtropical African climate between wetter and drier conditions orchestrated by the Earth orbital variations were marked by step-like (-0.2 or 0.4 Ma) increases in African climate variability and aridity near 2.8 Mya, 1.7 Mya, and 1.0 Mya, which coincide with the onset and intensification of high-latitude glacial cycles and variation in the degree of orbital precession (Vrba et al. 1989; deMenocal 1995; Clemens et al. 1996; Trauth et al. 2005).

Except for *Cryptospiza reichenovii*, genetic (phylogeographic) structure within the study models can be traced back to the late Pliocene. This result does not support the Pleistocene forest refugia as primary driver of diversification (Mayr & O'Hara 1986) but it does support the importance of Pliocene refugia (Voelker et al. 2010) that continued to play a role in the Pleistocene. The estimated divergence times coincide with the major Arctic glaciation that started at 3.15 Mya and culminated at 2.74 Mya, resulting in a gradual global cooling (Bartoli et al. 2005) that may have created the same corridors observed in the SDMs during the last glacial period. The first splits for *Bradypterus lopezi* and *Crithagra burtoni* comprise the separation between some Eastern Archipelago haplotypes and the western ones, which reappear later in the Pleistocene mixed in the western clade, suggesting episodes of migration and recolonization during the glacial periods.

Analyzing the phylogenies, the 2.8 Mya aridity period referred above affected *Pseudoalcippe abyssinica* and *Sheppardia bocagei* by interrupting the connection between Angola and the other centres, isolating the individuals until the present days.
The 1.7 Mya aridity period had broader consequences, having affected *Bradypterus lopezi*, *Cryptospiza reichevovii*, *Pseudoalcippe abyssinica* and *Sheppardia bocagei* by contributing to a clear split between eastern and western haplotypes. This was most certainly the consequence of the formation of an 'arid corridor' from 'Cape to Cairo' that

interrupted the connection between the western and the eastern centres (deMenocal 2004).

In the case of *Crithagra burtoni*, a similar split between the eastern and the western haplotypes can be observed soon after the other related episode of intense aridization of the continent around 1 Mya. The large haplotype divergence within Tanzania, and within the same locality (Kisinga Lugalo) is indicative of a recent recolonization of Tanzania suggesting that different colonisation events may have contributed to the assembly of the Afromontane bird communities – and may have sometimes interrupted the speciation process of lineages previously isolated. All of these late splits can be explained by the Pleistocene forest refugia hypothesis (Mayr & O'Hara 1986).

The presence of probable cryptic species can also be inferred in some cases. In *Bradypterus lopezi*, the level of divergence between the Uganda haplotypes and all the other samples points to the existence of a putative new species. The same scenario can be observed for the Angola haplotypes of *Pseudoalcippe abyssinica* and *Sheppardia bocagei*, which present a great and ancestral divergence from the other haplotypes.

Even though the splits occur in a different sequence across different species, and in cases like *Bradypterus lopezi*, *Crithagra burtoni* and *Cryptospiza reichenovii* some haplotypes from different locations appear in the same clade, it is clear that the splits between different geographical areas occurred around the same time intervals, and all these major splits cover the three time marks described previously, all linked to an increase in aridity and the formation of the 'arid corridor' splitting the eastern and western forests.

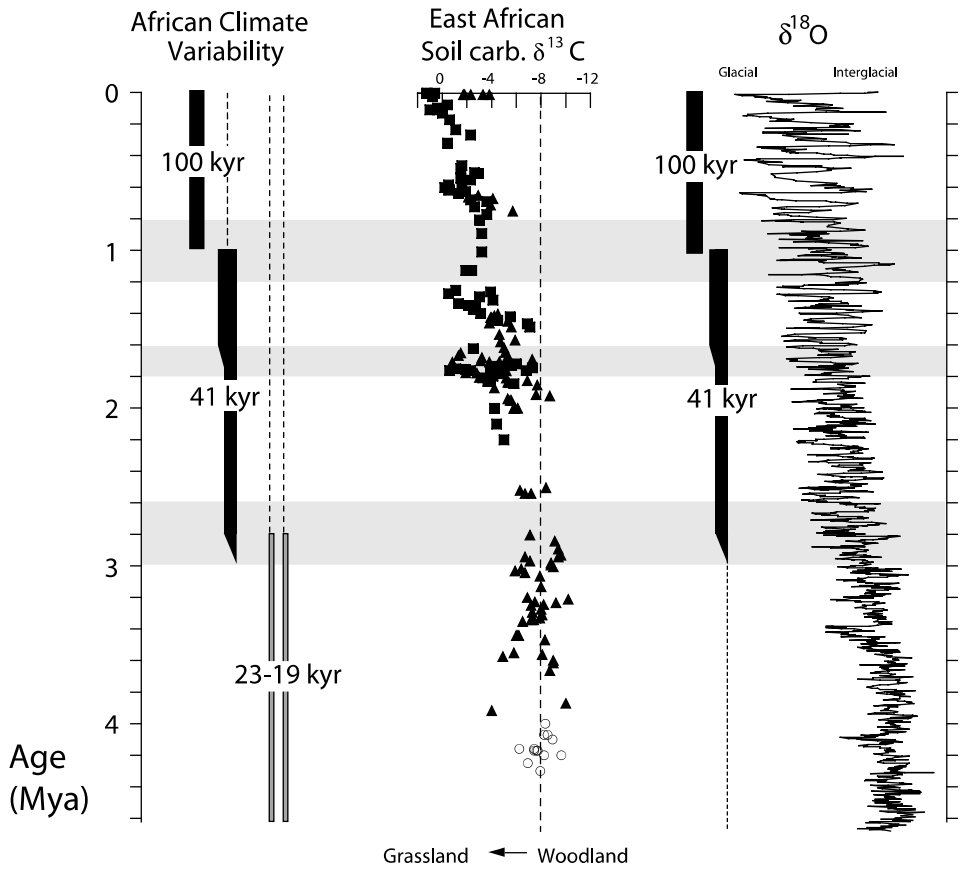

Figure 7 Summary diagram of important paleoclimatic events during the Pliocene-Pleistocene. Marine paleoclimatic records indicate that African climate became progressively more arid after step-like shifts (grey bands) near 2.8 Mya, and subsequently after 1.7 Mya and 1.0 Mya, coincident with the onset and intensification of high-latitude glacial cycles. Soil carbonate carbon isotopic data document the Pliocene-Pleistocene progressive shifts from closed woodland forest C3 to arid-adapted C4-pathway savannah grassland. Data adapted from deMenocal (2004).

Although the ecological stability of the different centres varied from species to species, Angola came up as a very stable centre for 4 out of 5 species. The stability of Angola may have played an important role in keeping populations of Afromontane species that went extinct elsewhere. The most ancient haplotypes occur for *Sheppardia bocagei* and *Pseudoalcippe abyssinica*. In the former case, results from the dynamic refugia approach supports Angola as a refuge from where the other centres were colonised. Direction of migration is difficult to infer for *Pseudoalcippe abyssinica* as all the three centres show areas of high suitability. The same occurs with *Bradypterus lopezi* and *Cryptospiza reichenovii*, whose most ancient haplotypes occur in the Eastern Archipelago. For *Crithagra burtoni*, which also presents the most ancient haplotypes in the Eastern Archipelago, this centre presents the highest suitability values, with Angola presenting low and restricted overall stability, suggesting that the migratory direction went from the Eastern Archipelago to the Cameroon centre via Angola.

Conclusions

This work produced the first molecular-based models of the historical links between all the major western and eastern centres of montane endemism in Africa. Overall the most frequent corridors were clearly between the east and Angola, which is reflected today in a larger share of conspecifics and closely related species between these two centres (Dowsett 1986). On the other hand, our five species demonstrated that the relations between the different centres were more diverse and complex than thought before. Examples of this diversity are represented by the multiple colonisations inferred in the *Crithagra burtoni* phylogeny (which added genetic diversity but interrupted speciation), or by some lines on hidden diversity/differentiation that were uncovered by this study, as the presence of probable cryptic species in the phylogenies of *Bradypterus lopezi*, *Pseudoalcippe abyssinica* and *Sheppardia bocagei*.

This study used birds to present the first ever picture of the evolutionary relationships of communities within the Afromontane archipelago. Angola, a neglected Afromontane Centre (due to its small size, isolation, and lack of access due to four decades of armed conflict) is not just a missing piece of the Afromontane puzzle, but a fundamental piece. Angola has acted as both as a very stable centre – which played an important role as a refuge from where refuge populations could recolonize other areas when climatic conditions became more favourable – and as a stepping-stone linking the East Africa and the Cameroon Centres.

To confirm these results and gain further insights, it will be important to achieve a denser taxon sampling (more individuals per locality and more localities) and obtain a larger genetic dataset, including markers from the nucleus – independent from the mitochondrial markers used here.

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Attachments

Attachment 1a *Bradypterus lopezi* samples with code and country for individual specimens, with respective voucher and GenBank accession numbers of both ATP6 and ND2 sequences used in this study. Acronyms are: CIBIO - Research Centre in Biodiversity and Genetic Resources; UCB - University of California, Berkeley.

Code	Country (location)	Voucher samples	ATP6	ND2	Source
BRA_A01	Angola (Namba)	CIBIO A3-041	-	-	-
BRA_C01	Cameroon (Mann's Spring)	CIBIO C090	-	-	-
BRA_C02	Cameroon (Mann's Spring)	CIBIO C142	-	-	-
BRA_C03	Cameroon (Mann's Spring)	CIBIO C168r	-	-	-
BRA_C04	Cameroon (Mount Cameroon)	UCB RB4012	-	-	-
BRA_C05	Cameroon (Mount Cameroon)	UCB RB4013	-	-	-
BRA_C06	Cameroon (Mount Cameroon)	UCB RB4014	-	-	-
BRA_G01	Eq. Guinea (Basilé Peak)	CIBIO B028	-	-	-
BRA_G04	Eq. Guinea (Basilé Peak)	CIBIO B092	-	-	-
BRA_G05	Eq. Guinea (Basilé Peak)	CIBIO B145	-	-	-
BRA_K01	Kenya (Kenyan Highlands)	UCB RB1817	-	-	-
BRA_K02	Kenya (Kenyan Highlands)	UCB RB1833	-	-	-
BRA_M01	Malawi (Zomba)	CIBIO MW-CS04	-	-	-
BRA_M02	Malawi (Zomba)	CIBIO MW-CS05	-	-	-
BRA_M03	Malawi (Southern Malawi Rift Mts.)	UCB RB4019	-	-	-
BRA_M04	Malawi (Southern Malawi Rift Mts.)	UCB RB4021	-	-	-
BRA_M05	Malawi (Southern Malawi Rift Mts.)	UCB RB4022	-	-	-
BRA_T01	Tanzania (Eastern Arc Mountains)	UCB KMN107	-	-	-
BRA_T02	Tanzania (Eastern Arc Mountains)	UCB KMN108	-	-	-
BRA_T03	Tanzania (Eastern Arc Mountains)	UCB RB3535	-	-	-
BRA_T04	Tanzania (Eastern Arc Mountains)	UCB RB3536	-	-	-
BRA_T05	Tanzania (Nyika Plateau)	UCB RB4015	-	-	-
BRA_T06	Tanzania (Nyika Plateau)	UCB RB4016	-	-	-
BRA_U01	Uganda (Ruwenzori Mountains)	UCB SS102	-	-	-
BRA_U02	Uganda (Ruwenzori Mountains)	UCB SS103	-	-	-

Attachment 1b *Crithagra burtoni* samples with code and country for individual specimens, with respective voucher and GenBank accession numbers of both ATP6 and ND2 sequences used in this study. Acronyms are: CIBIO - Research Centre in Biodiversity and Genetic Resources; UCB - University of California, Berkeley; MNHN - Museum National d'Histoire Naturelle; NRM - Swedish Museum of Natural History, Stockholm; ZMUC - Zoological Museum of the University (Copenhagen).

Code	Country (location)	Voucher samples	ATP6	ND2	Source
CRI_A01	Angola (Moco)	CIBIO A1-096	-	-	-
CRI_A02	Angola (Moco)	CIBIO A3-019	-	-	-
CRI_C01	Cameroon (Mbouroukou)	MNHN 40-55	EU880954	-	Nguembock et al. 2009a
CRI_C02	Cameroon	NRM 20086267	-	JN715479	Zuccon et al. 2012
CRI_C03	Cameroon (Mann's Spring)	CIBIO C084	-	-	-
CRI_C04	Cameroon (Mann's Spring)	CIBIO C119	-	-	-
CRI_DRC01	DR Congo (Tshibati Fort)	ZMUC 128777	EU880976	-	Nguembock et al. 2009a
CRI_K01	Kenya (Karissia Hills)	ZMUC 131436	EU880966	-	Nguembock et al. 2009a
CRI_N01	Nigeria (Obudu Plateau)	UCB FB26320	-	-	-
CRI_N02	Nigeria (Obudu Plateau)	UCB FB26325	-	-	-
CRI_T01	Tanzania (Nduldulu Mountains)	ZMUC 118807	EU880977	-	Nguembock et al. 2009a
CRI_T02	Tanzania (Kisinga Lugalo)	ZMUC 118856	EU880978	-	Nguembock et al. 2009a
CRI_T03	Tanzania (Kisinga Lugalo)	ZMUC 134568	EU880979	-	Nguembock et al. 2009a
CRI_T04	Tanzania (Nou Forest)	ZMUC 134797	EU880995	-	Nguembock et al. 2009a
CRI_T05	Tanzania (Salanga Forest)	ZMUC 130427	EU880996	-	Nguembock et al. 2009a
CRI_T06	Tanzania (Udzungwa Scarp)	ZMUC 123647	EU880997	-	Nguembock et al. 2009a

Attachment 1c *Cryptospiza reichenovii* samples with code and country for individual specimens, with respective voucher and GenBank accession numbers of both ATP6 and ND2 sequences used in this study. Acronyms are: CIBIO - Research Centre in Biodiversity and Genetic Resources; UCB - University of California, Berkeley; MNHN - Museum National d'Histoire Naturelle; NRM - Swedish Museum of Natural History, Stockholm; ZMUC - Zoological Museum of the University (Copenhagen).

Code	Country (location)	Voucher samples	ATP6	ND2	Source
CRY_A01	Angola (Namba)	CIBIO A3-052	-	-	-
CRY_A02	Angola (Kumbira)	CIBIO A4-020	-	-	-
CRY_A03	Angola (Kumbira)	CIBIO A4-021	-	-	-
CRY_A04	Angola (Kumbira)	CIBIO A4-051	-	-	-
CRY_A05	Angola (Kumbira)	CIBIO A6-2	-	-	-
CRY_AR01	Albertine Rift	UCB FM346704	-	-	-
CRY_AR02	Albertine Rift	UCB FM356444	-	-	-
CRY_C01	Cameroon	UCB SAN951214	-	-	-
CRY_C02	Cameroon (Buea)	CIBIO C177	-	-	-
CRY_C03	Cameroon	MNHN	JX259158	JX259206	Nguembock et al. 2012
CRY_C04	Cameroon	MNHN 42-21	-	EU686332	Nguembock et al. 2009b
CRY_EA01	Tanzania (Eastern Arc Mountains)	UCB JK06_200200	-	-	-
CRY_EA02	Tanzania (Eastern Arc Mountains)	UCB JK07_240300	-	-	-
CRY_M01	Malawi (Zomba)	CIBIO MW-CS03	-	-	-

Attachment 1d *Pseudoalcippe abyssinica* samples with code and country for individual specimens, with respective voucher and GenBank accession numbers of both ATP6 and ND2 sequences used in this study. Acronyms are: CIBIO - Research Centre in Biodiversity and Genetic Resources; UCB - University of California, Berkeley; MNHN - Museum National d'Histoire Naturelle.

Code	Country (location)	Voucher samples	ATP6	ND2	Source
PSE_A01	Angola (Moco)	CIBIO A1-057	-	-	-
PSE_A02	Angola (Moco)	CIBIO A1-060	-	-	-
PSE_A03	Angola (Moco)	CIBIO A1-073	-	-	-
PSE_A04	Angola (Moco)	CIBIO A3-033	-	-	-
PSE_AR01	Albertine Rift	UCB RB3098	-	-	-
PSE_AR02	Albertine Rift	UCB RB3099	-	-	-
PSE_C01	Cameroon (Mount Cameroon)	UCB GA59565	-	-	-
PSE_C03	Cameroon (Northwest Region)	UCB kyn113	-	-	-
PSE_C04	Cameroon (Northwest Region)	UCB kyn128	-	-	-
PSE_C05	Cameroon (Northwest Region)	UCB kyn164	-	-	-
PSE_C06	Cameroon (Mount Cameroon)	MNHN 40-75	JX259154	EU652717	Nguembock et al. 2012
PSE_EA01	Tanzania (Eastern Arc Mountains)	UCB RB2864	-	-	-
PSE_G01	Eq. Guinea (Bioko)	UCB GA59742	-	-	-
PSE_G02	Eq. Guinea (Bioko)	UCB GA59760	-	-	-
PSE_G03	Eq. Guinea (Bioko)	UCB GA59763	-	-	-
PSE_K01	Kenya	UCB RB3032	-	-	-
PSE_K02	Kenya	UCB RB3033	-	-	-
PSE_K03	Kenya	UCB RB3034	-	-	-

Attachment 1e *Sheppardia bocagei* samples with code and country for individual specimens, with respective voucher and GenBank accession numbers of both ATP6 and ND2 sequences used in this study. Acronyms are: CIBIO - Research Centre in Biodiversity and Genetic Resources; UCB - University of California, Berkeley.

Code	Country (location)	Voucher samples	ATP6	ND2	Source
SHE_A01	Angola (Moco)	CIBIO A1-053	-	-	-
SHE_A02	Angola (Moco)	CIBIO A1-054	-	-	-
SHE_A03	Angola (Moco)	CIBIO A1-055	-	-	-
SHE_A05	Angola (Moco)	CIBIO A6-51	-	-	-
SHE_A06	Angola (Tundavala-Lubango)	CIBIO A2-005	-	-	-
SHE_G01	Eq. Guinea (Moka)	CIBIO B207	-	-	-
SHE_G02	Eq. Guinea	-	-	FN546907	Voelker et al. 2010
SHE_G03	Eq. Guinea	-	-	FN546908	Voelker et al. 2010
SHE_T01	Tanzania	UCB JP916	-	-	-
SHE_T02	Tanzania	UCB JP917	-	-	-

Attachment 2 Primers and PCR conditions for the markers used in this study.

Primers	Sequence	References	PCR-mix				PCR-cycle					
			DNA (µM)	Taq (µM)	Primer (µM)	dH2O (µM)	[PREMIT]	[DENAT	ANNEAL	EXT]	CYCLES	FINAL
ATPase6 (L9245)	5'-CCTGAACCTGACCATGAAC-3'	http://striweb.si.edu/bermingham/	2	3	0.6	8.8	95ºC-15'	95ºC-30''	63/59ºC-30''	72ºC-75''	10+27	72ºC-10'
ATPase6 (H9947)	5'-CATGGGCTGGGGTCRACTATGTG-3'	http://striweb.si.edu/bermingham/										
ND2 (L5215)	5'-TATCGGGCCCATACCCCGAAAAT-3'	Hackett 1996					95ºC-15'	95ºC-30''	56ºC-30''	72ºC-90''	37	72ºC-10'
ND2 (H1064)	5'-CTTTGAAGGCCTTCGGTTTA-3'	Drovetski et al. 2004										

Attachment 3 Bioclimatic variables used for the niche modelling.

Code	Correspondence
BIO1	Annual Mean Temperature
BIO4	Temperature Seasonality (standard deviation *100)
BIO10	Mean Temperature of Warmest Quarter
BIO11	Mean Temperature of Coldest Quarter
BIO12	Annual Precipitation
BIO15	Precipitation Seasonality (Coefficient of Variation)
BIO16	Precipitation of Wettest Quarter
BIO17	Precipitation of Driest Quarter

Attachment 4 Estimated divergence times with respective 95% intervals for each species.

Species	TMRCA	Full Dataset 2 genes	Monospecific 2 genes	Monospecific ATP6	Monospecific ND2
Bradypterus lopezi	4th Split	0.23-0.35-0.49	0.23-0.35-0.49	na	na
	3rd Split	0.58-0.78-1.00	0.58-0.78-1.01	na	na
	2nd Split	1.13-1.57-2.04	1.07-1.49-1.95	na	na
	1st Split	2.47-3.07-3.78	2.42-3.13-3.86	na	na
Cryptospiza reichenovii	2nd Split	0.09-0.15-0.20	na	na	na
	1st Split	1.18-1.54-1.93	na	na	na
Crithagra burtoni	3rd Split	0.20-0.36-0.52	0.17-0.32-0.47	0.16-0.32-0.49	na
	2nd Split	0.57-0.77-0.98	0.52-0.72-0.93	0.47-0.76-1.08	na
	1st Split	1.85-2.37-2.90	1.96-2.67-3.47	1.77-2.53-3.48	na
Pseudoalcippe abyssinica	4th Split	0.72-0.94-1.17	na	na	0.78-1.07-1.40
	3rd Split	0.79-1.01-1.27	na	na	0.86-1.17-1.52
	2nd Split	1.06-1.38-1.70	na	na	1.16-1.60-2.09
	1st Split	2.73-3.46-4.16	na	na	5.30-8.03-11.25
Sheppardia bocagei	2nd Split	0.82-1.25-1.73	0.77-1.24-1.76	na	na
	1st Split	2.03-2.59-3.19	2.16-2.88-3.65	na	na